

Investigation of the Concentration Polarization effect on Nanoparticles using radial micro-nanofluidic device

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ABSTRACT

We have developed a silicon micro-nanofluidic devices in order to investigate a strong nanofluidic phenomenon called "Ion Concentration Polarization" (ICP). The device used were, called "Ring like" device, exhibits a circular micro-nanojunction. Nanochannels, 100nm in depth, were fabricated by standard photolithography and etching techniques. Ion perm-selectivity is one of the major proprieties of nanofluidic devices. Within the influence of an electric field through an ion-selective nanochannel, ion depletion, at the anodic side, and ion concentration at the cathodic side, appear. Here, we show that 50nm polystyrene nanobeads can be depleted and concentrated using ICP. The device could be use to concentrate biological nanoparticles like viruses (from tens to hundreds of nanometers), without the risk of nanochannel clogging as no pressure field is induced.

Keywords: Radial nanochannels, Ion Concentration Polarization, nanobeads.

1 INTRODUCTION

With the advances of Micro and Nanofabrication techniques, science of particles and molecular transport within Nanofluidic Systems has drawn more and more attentions. Ion perm-selectivity can be seen as one of the major proprieties of nanofluidic devices. Because the Debye layer thickness (λ_D) is non-negligible compared with the nanochannel thickness, electrical double layer can overlap inside the nanochannels under certain conditions. As a consequence, nanochannels become ion perm-selective. Within the influence of an electric field through the nanochannels, a phenomenon called ICP "Ion Concentration Polarization" [1] appears, creating near the micro-nanojunction an ion depletion/concentration zone at the anodic/cathodic side, respectively. To satisfy electro neutrality, the depletion zone behaves as an electrostatic barrier to any charged particle. As a consequence, this zone can be use as a powerfull tool for strong concentration of charged analytes. Recent works have shown that microfluidic-nanofluidic interfaces can be used as

preconcentration devices that have a important impact on molecules such as fluorescein or proteins (up to million fold[2]). However, more investigations of this phenomenon have to be made on particles larger than molecules or macromolecules, such as nanoparticles which can mimic biological species like viruses (from tens to hundreds of nanometer). Also, regarding concentration polarization based nanofluidic concentrators, nanochannels or nanoporous membranes, such as nafion, were commonly used as planar nanofluidic junction connecting microchannel following the classical "Bypass" configuration [1-2]. Scarff et al [3] have proposed an original radial sample preconcentration strategy using circular Nafion nanojunction. This radial configuration offers several advantages such as: (i) non-linear field effects that confine the sample to a small central region; (ii) increased interfacial area and increased ionic currents and; (iii) no balancing flows are required as compared to the usual axial geometry. Scarff et al [3] have demonstrated proof of concept FITC-BSA concentration in the central microfluidic chamber in the center of the circular nanojunction using the expansion behavior of ion depletion. Concentration as high as 168 times the initial concentration have been demonstrated using this device. Here, we perform ICP using radial silicon nanojunction geometries fabricated by single standard silicon microfabrication for proof of concept concentration operation over 50nm polystyrene nanobeads. We call the device the "Ring like devices" because of the circular shape of the nanojunction. The ion-selective nanojunction exhibits 4 radial nanochannels. Unlike previous concentration polarization based schemes; using the Ring like device, our concentration process relies partly on the concentration zone that occurs at the cathodic side of the nanojunction in the circular micro chamber at the periphery of the circular nanochannel. Ion concentration zone allow for continuous concentration of nanobeads up to ~1000 times the initial concentration over 1 hour. The concentration process is very stable and does not require any backflow for concentration operation. The strong and stable concentration effect of fluorescent nanoparticles is of special importance when it comes to consider the early detection of biological species such as viruses for instance.

Ion perm-selectivity is one of the major properties of fluidic channels of the nanoscale (<100nm). When a solid surface is immersed in a liquid, an electrical double layer called the Debye Layer will appear to screen the solid surface charge. The thickness (λ_D) of the electrical double layer will depend on the conductivity of the liquid. The lower the conductivity is, the higher will be λ_D . If we consider a nanochannel (with at least one dimension <100nm) immersed in a liquid and an electrical double layer with a thickness comparable to the channel dimension, the double layers will overlap inside the nanochannel. This will lead to a partial screening of the surface potential of the solid wall and to a selective permeability to ions (figure 1 a-b-c). Co-ions (ions with the same charge as the wall surface charge) will be prohibited from the nanochannel by electrostatic repulsion whereas counter ions (ions with opposite charge compared to the wall surface charge) will be attracted inside the nanochannel.

Figure 1 shows a simplified mechanism of the ICP phenomenon according to the most reported explanation in the literature.

When an electric field is applied across a nanochannel, which makes the bridged between two microchannels, counter-ions (positive ions when the surface charge of the wall is negative) will be actively pumped through the nanochannel from the anode toward the cathode. In order to rebuild the electro neutrality locally broken (figure 1c) near the micro-nanojunction, co-ions will leave the anodic side and repopulate the cathodic side. This will lead to a depletion and enrichment of both co and counter-ions at the anodic and cathodic side, respectively (figure 1d).

This phenomenon has already proven its great potential for various applications. Indeed, because the depletion zone acts as an electrostatic barrier where charge analytes can accumulate or can be deviated while applying an external force (pressure or electrical) without using any added component (mechanical sieve or magnetic beads, for instance), this phenomenon has shown great performances in detection (high concentration fold [2]). However, more studies have to be done for full understanding of this phenomenon.

2 RESULTS AND DISCUSSIONS

First, we propose to compare the influence of ICP phenomenon on molecules and nanoparticles, whereas most of the studies focused preferentially on molecules like proteins, for instance. Also, it is an important issue to explore the influence of different geometry of the micro-nanojunction on ICP performances.

A single ring like device composed of a circular micro-nanojunctions, was fabricated by standard silicon fabrication steps. Figure 2 shows the fabrication steps of the single device and the top view of the circular micro-nanojunction. The radial nanofluidic channels (100nm in

height), the microchannels (15 μ m in depth) and through-holes (600 μ m in diameter) were defined and etched into a 100 mm silicon wafer using photolithography and reactive-ion etching technique. A profilometer was used to carefully measure the channel height (at step c) which is the only critical dimension of our chip. A good uniformity of 100 \pm 5nm was measured on the overall 100mm wafer (figure 1-B).

Figure 3 shows the effect of anodic depletion on fluorescein and 50nm nanobeads in the Ring like device.

At $t=0$, no electric field was applied across the nanochannels. Fluorescence of fluorescein and fluorescent nanobeads was homogenous in the entire sample microchannel in the Bypass and the Ring like device.

One minute after a 50V potential drop was applied across the nanochannels, we observed the depletion of both fluorescein and nanobeads represented by a strong decrease of fluorescence inside the sample microchannel. Because of the quick extension of the depletion zone in the microchannels, several minutes after ignition of the ICP phenomenon, the fluorescence intensity has completely disappeared from the microchannel because all the fluorescent molecules and nanobeads were repealed toward the outlets. The depletion zone is an electrostatic barrier which prohibited any charged particle, including salts, to go through. Then, we can consider that, a few minutes after ignition of Concentration Polarization, completely pure and desalted water remained in the sample microchannel. Extension speed of the depletion zone was the same for both fluorescein and nanobeads and for each device. An estimation of \sim 0.5mm/min was measured. A characteristic "depletion shock", represented by a local enrichment of fluorescein and fluorescent nanobeads, located at the boundaries of the depletion zone was observed. Even if similar depletion behaviours are shown, the enrichment of fluorescein at the depletion boundaries was more significant compared to the nanobeads and the shape of the depletion shocks was slightly different in each case. Additionally, the fluorescent microbeads mixed with fluorescein and fluorescent nanobeads were also repealed during the anodic depletion process but some were trapped inside the depletion zone and revealed the presence of strong vortices near the micro-nanojunction.

In Figure 4 50 nm nanobeads were concentrated at the cathodic side in the peripheric circular microfluidic chamber for 1h. We call the concentration process "Cathodic Concentration" (CC). As mentioned above, once an electric field is established through perm-selective nanochannels, ICP phenomenon occurs and allow for the enrichment of any charged particules near the nanojunction at the cathodic side when the surface charge of the nanochannel is negative (if the surface charge of the nanochannel was positive, the enrichment effect would occur at the anodic side of the nanochannel).

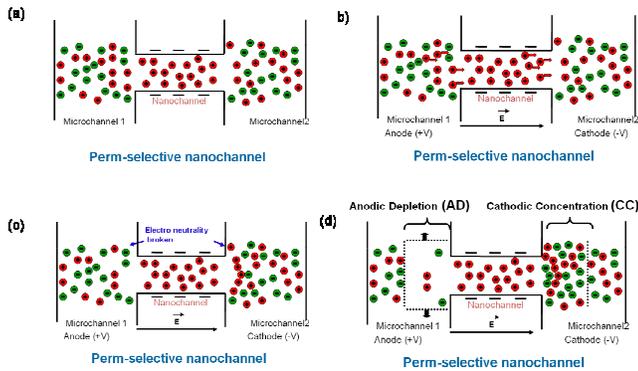


Figure 1: Mechanism of ICP in a bypass device (two parallel microchannels bridged by one (or more) nanochannel). (a) At t_0 no voltage is applied. (b)-(c)-(d) When a voltage is applied across the nanochannel, counterions will be actively pumped through the nanochannel (positive ions when the surface charge of the wall is negative) from the anode toward the cathode. In order to rebuild the electro neutrality locally broken near the micro-nanojunction, co-ions will leave the anodic side and repopulate the cathodic side. This will lead to a depletion and enrichment of both co and counter ions at the anodic and cathodic side, respectively.

Steps	Bypass device	Ring like device
(A) Silicon Wafer (100mm)	Si	Si
(B) 100nm nanochannels etching (RIE)		
(C) Thermal hard mask patterning (RIE)		
(D) Partial etching of access holes (DRIE)		
(E) 15 μ m microchannels etching + final etching of access holes (DRIE)		
(F) Thermal oxide + silica/glass(500 μ m) molecular bonding	Borofloat	Borofloat

Figure 2 : Schematic description of Ring like device fabrication steps on silicon substrate as compared to a classical bypass device. In the case of the ring like device an additional access hole is etched. (A) The nanofluidic channels (100nm in height), the microchannels (15 μ m in depth) and through-holes (600 μ m in diameter) were defined and etched into a 100 mm Si wafer using photolithography and reactive-ion etching technique. (B) First, the nanochannels are patterned and etched by contact photolithography and reactive-ion etching. (C) A 500nm thick thermal silicon dioxide layer was grown. The microchannel was patterned in the thermal oxide layer using Reactive Ion Etching (RIE). (D) Following the hard mask etching, through holes were etched using

photolithography and Deep-RIE. (E) Microchannel is then etched through the hard mask using DRIE. (F) After removing the thermal oxide (hard mask) and cleaning the substrate, a second thermal oxide layer (300 nm thick) was grown to provide electrical isolation. Finally, patterned silicon wafer and glass cover (Borofloat wafer, 0.5 mm thick) were bonded by a molecular bonding technique.

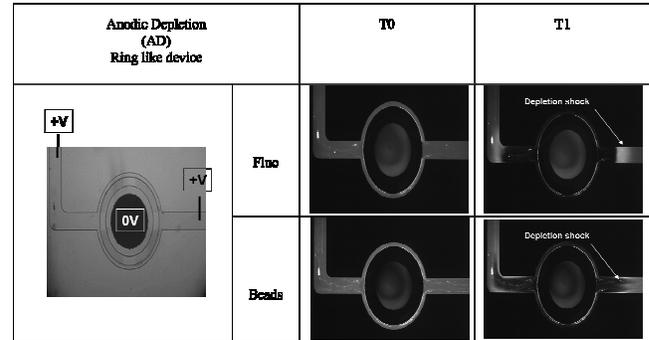


Figure 3: Anodic depletion of 50nm fluorescent polystyrene nanobeads and fluorescein using the new Ring like device. $V=50V$. A depletion speed of $\sim 0.5\text{mm/min}$ was estimated for, both fluorescein and nanobeads

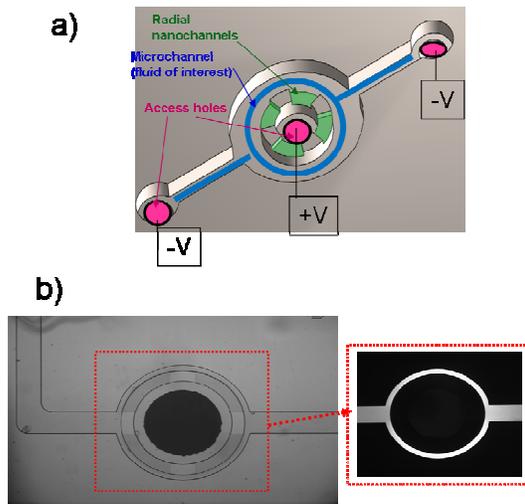


Figure 4: Cathodic concentration of 50nm nanobeads in the single ring like device. (a) 3D scheme of the single Ring like device. The center hole is connected to the anode while the peripheral holes are connected to the cathode so that the concentration of the nanobeads occurs in the microchannel. (b) top view of the radial nanojunction during the concentration process. Observation of the 50nm fluorescent beads by fluorescent imaging. Up to 1000 fold can be obtained after 1h.

3 MATERIAL AND METHODS

3.1 Materials and Reagents

The buffer, deionised water (18.2 M Ω .cm @ 25°C, 10⁻⁶M), was used for all experiments to facilitate the electrical double layer overlap inside the 100nm deep nanochannels. 50nm green fluorescent polystyrene nanobeads (INTERTEK, excitation maxima: 468nm, emission maxima: 508nm) and fluorescein (SIGMA Aldrich, excitation maxima: 490nm, emission maxima: 514nm) were used to visualize the depletion zone. A 10¹² pl/ml concentration of nanobeads was used for all experiments. Zeta potentials of the nanobeads were evaluated using a zeta sizer in DI water. Values of -66 mV were measured. To control and visualize the flow inside the device, 1 μ m red Fluorescent polystyrene microbeads (INTERTEK, excitation maxima: 542nm, emission maxima: 612nm) were added to the nanobeads and fluorescein solutions.

3.2 Measurement Instruments

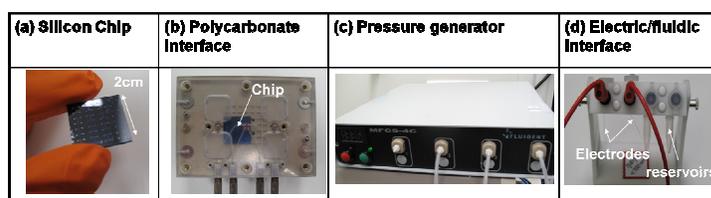
All the experiments were imaged with an epifluorescence microscope (Olympus, BX60M), which has a built-in 100W mercury lamp. A standard FITC (Olympus, Melville, NY) and homemade filter set were used for the detection of green and red emissions, respectively. In these experiments, CCD camera (MIKROTRON, MotionBlitz EoSens cub 7) was used for fluorescence imaging. Sequences of images were analyzed by Image J.

3.3 Experimental setup

First, the sample channel was filled with micro-nanobeads mixture and the buffer channel was filled with deionised water using a pressure generator (MFCS 4C, FLUIGENT). The pressure generator was preferable than a syringe pump because of its better stability. Motion of microbeads was then visualized to detect when the flow is stabilized inside the sample microchannel. To initiate the anodic depletion in the sample microchannel, a voltage of 50V was applied. The voltage was generated in the liquid, thanks to a voltage generator (Stanford research System, INC., Model PS 350/ 5000V-25W) and platinum electrodes (Electrowell, FLUIGENT) immersed in the sample and the buffer reservoirs. Waterproofness was ensured with a 1cm polycarbonate interface (From PMP).

Resume of the methodology:

- 1) Injection of fluorescent solutions (fluorescein or nanobeads) and DI-water in the sample and buffer channel, respectively
- 2) Stabilization of the flow
- 3) Ignition of the anodic depletion at the anodic side
- 4) Images acquisition



CONCLUSION

We have shown the first evidence of the impact of ICP on nanoparticles using a radial micro-nanofluidic device with 100nm deep nanochannels. Both depletion and concentration of 50nm polystyrene beads have been demonstrated. Unlike previous concentration polarization based schemes; using the Ring like device, our concentration process relies on the ion concentration phenomenon that occurs at the cathodic side of the nanojunction in the circular micro chamber at the periphery of the circular nanochannel. Ion concentration zone allow for continuous concentration of nanobeads over a long period of time (more than 1 hour) and does not require any backflow for concentration operation. This is of special importance as it may open the door for further investigations including the concentration of viruses for lab on chip applications.

REFERENCES

- [1].Pu,Q. Jongsin Yun, Henryk Temkin, et Shaorong Liu, 2004. *Nano Letters* 4, 1099–1103.
- [2].Wang, Y.-C., Stevens, A.L., Han, J., 2005. *Analytical Chemistry* 77, 4293–4299.
- [3] Scarff, B., Escobedo, C., Sinton, D., 2011. *Lab on a Chip* 11, 1102–1109.